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Visualization of sample introduction in liquid chromatographic columns
Contribution of a flow distributor on the sample band shape

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Abstract

The contributions to the radial distribution of the sample concentration across the column inlet and to the axial band dispersion resulting from a column header containing a distributor were evaluated using a band-visualization process entailing matching the refractive indices of the stationary and mobile phases in a glass column. This study illustrates graphically how a distributor fitted to the column can increase the axial dispersion of the sample band compared to an inlet containing only a frit. The distributor did not provide a uniform sample distribution across the column. In fact, for 17-mm inner diameter columns and high-porosity frits, the distribution was no better than with the frit having no distributor. However, when low-porosity frits were employed, improved peak shapes were obtained with a distributor. In addition, we observed that the inlet header configuration influenced dramatically the flow stream established along the column. The radial distribution of the efficiency of the columns was nearly homogeneous for those having only a frit but not for those having also a distributor. For the latter, the efficiency decreased from the column axis to its wall.

Keywords: Visualization; Sample introduction; Band profiles; Flow distributor

1. Introduction

In a previous communication, we illustrated the influence of the inlet frit on the column performance [1]. This study was performed using an on-column visualization technique that allowed viewing the sample band directly and entirely, photographing it and converting it into a concentration distribution across the column bed. This method was previously discussed in detail [1–4]. The importance of acquiring data regarding the three-dimensional structure of the band was demonstrated. This was needed, for example, to recognize cases in which the mobile phase stream entering the column bypassed a low permeability frit by flowing around its edge, along the wall, or cases in which the injection was not coaxial with the column [1].

We also showed that the frit permeability should not be too low but should match that of the bed. With the packing material (20–30 μm particles) used, the larger 10 μm pore-diameter frits produced a much better radial distribution of the sample across the

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column head than did the finer 2 μm frits [1]. The best of the 2 μm frits that we tried was only marginally better than the worst 10 μm frit. To achieve acceptable results with a 2 μm frit, extreme care had to be taken that the mobile phase stream did not bypass the frit. Even then, the concentration distribution in the sample band was parabolic, the band entering the column in the central region first and later in the wall region. Visualization of the sample band from two perpendicular directions revealed that the band was not symmetrical but that there were regions of the frit in which the flow velocity was higher or lower than average. Because of the parabolic profile of the injected band, peak broadening was more important than for the 10 μm frits, with which radial concentration distribution in the sample band was more homogeneous and flatter, with the exception of the region immediately near the wall.

Similar phenomena were previously identified and reported by other investigators [5–12]. They all demonstrate that any significant, systematic deviation from piston flow behavior of the chromatographic process, either for the lack of homogeneity of the bed or for a deviation from planarity of the injected band, results in a loss of column efficiency. Using local detection at the column outlet, Farkas and co-workers [5–7] showed that a large loss of efficiency was experienced when the radial distribution of the flow velocity was not flat. Similar results were obtained by Tallarek and co-workers [8,9], using nuclear magnetic resonance (NMR) imaging. With a similar method, Lode et al. [13] observed a band profile similar to one obtained by us with a 2 μm frit [1]. However, because the NMR technique used gives only two-dimensional information, it was not possible to study the uniformity of the injection profile across the column head. The authors concluded that another approach should be used to investigate the design of column headers. Hydrodynamic modeling seems to offer great promises in this area [14].

In a recent study on frit diameters [15] we illustrated the importance of matching the frit diameter to the internal diameter of the column [15]. If the frit diameter is smaller than the internal diameter of the column, a sharply parabolic injection profile was obtained; the smaller the frit diameter, the longer and narrower the injection profile. As a consequence, the column experienced a dramatic loss of performance. The profiles of the band entering the column through 2 μm frits [1] are milder forms of the profile resulting from a narrow frit [15]. This suggests that neither the distribution of the sample concentration nor that of the mobile phase flow velocity were homogeneous with low porosity frits.

While the use of a relatively large porosity frit seemed to improve the distribution of the band concentration across the head of the column, using such a frit places some limitations on the column design. In some instances, the use of a low porosity frit is essential. For example, one may need that the frit serves as a filter to prevent suspended solids in the sample to plug the bed around the column head. Although a pre-column or an in-line filter would prove more useful in this respect, the frit may serve as a last ditch protection. More importantly, the frit serves to hold the packing within the column and, in axial compression columns, to maintain the bed under compression. The inlet frit must prevent the particles from escaping. Accordingly, the average pore diameter of the frit must be smaller than that of the small particles in the packing material, so as to prevent frit obstruction. This only means, however, that the frit pore-size must be reduced in proportion to the average particle size of the packing (with possible allowance for differences in the particle size distribution).

The purpose of this work is to complete our investigation of the behavior of a sample band entering a column by studying the effects of sample distributors. Usually, chromatographers think little about inlet fittings and rarely use a distributor on an analytical column. Essentially, the frit serves to protect the head of the column, to keep the packing material from spilling over, and plays whatever role it may in the spreading of the injected band. It is the independent role of the distributor to improve the distribution of the sample concentration across the column head. This role becomes more important when larger diameter columns are employed.

2. Experimental

The chemicals, reagents, equipment and data analysis procedures used in this work were described in preceding communications [1–4,15] and further discussion is not warranted. All the experiments were performed on a 100×17 mm borosilicate (Pyrex) glass column (Omni, Cambridge, UK) with end fittings prepared by the departmental workshop, machined from Delrin plastic. The column was packed with YMC C_{18} silica (Kyoto-Fu, Japan), a spherical material with a particle size distribution given as 15–30 μm and an average particle size of 21 μm. The column was slurry packed in a downward configuration with methanol as both the slurry and the packing solvent. The mobile phase was pure carbon tetrachloride and the samples were solutions of iodine in the mobile phase (12 g/l).

New and relevant to this study is the information regarding the design of the distributor and its connection to the column inlet. The inlet assembly is shown in Fig. 1. It consists of a variable inlet head, the distributor and the inlet frit. The inlet head surface was flat, allowing the placement of the distributor level and flat on the inside edge. The inlet frit was then placed on the outlet side of the distributor. The whole assembly was bound tightly in PTFE tape, ensuring a leak proof seal on the outside edges of the distributor and the frit. The tape also provided a tight fit inside the column. This assembly was then inserted into the glass column. The head was tightened to a torque of 0.2 m·kg. The column was then allowed to sit overnight before testing. This provided a sufficient degree of bed consolidation and the obtention of an efficient column. Because of the design of the column, however, the inlet assembly could not be removed without destroying the packed bed. As a result, the packing of a new column was necessary for all other configurations tested.

The dimensions of the distributor were large for this type of column. The diameter was 16 mm, covering practically the whole internal diameter of the column. However, its thickness was 5 mm. Since the column is 100 mm long, the volume of the distributor is not completely negligible compared to that of the column. All injection volumes were 20 μl and all flow-rates were 1.5 ml/min.

Vertical scans parallel to the column axis were made at various radial positions on photographs of the band taken at various migration times. The migration distances for each scan at each radial position were determined from the maxima of each concentration profile. The normalized migration dis-

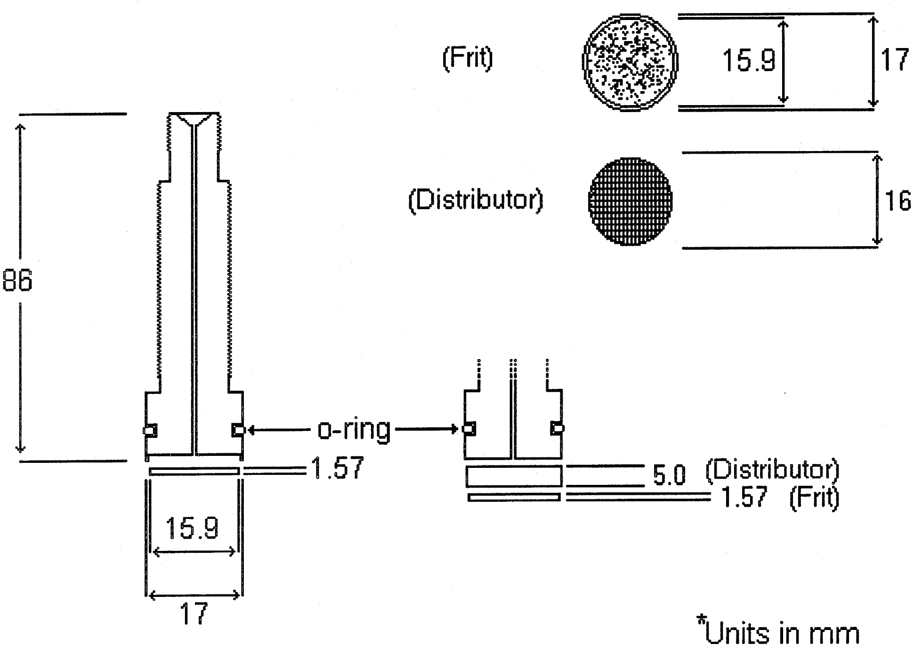

Fig. 1. Diagram of the column header. (a) Frit, (b) distributor.

tance was determined by dividing each of these migration distances by the migration distance of the band measured at the column center. This procedure was repeated for each photo taken and analyzed in this work.

3. Results and discussion

The design of the distributor used in this work was that of a decreasing meshed wire sieve. The upstream side had a coarse mesh while the packing side was fine. The principle is to allow the solvent entering the frit to disperse rapidly through the larger mesh at the entrance and then to be more finely divided by the narrow mesh closer to the bed. To evaluate the effectiveness of the distributor, we studied the chromatographic behavior of iodine under NARP conditions, using the distributor by itself and combined with the best of the 2 μm frits or a 10 μm frit, which were both used previously [1]. We should note at this point, however, that the distributor employed in this study was principally designed for sample distribution on a wide diameter column – in the order of 5 cm. The column used in this study was only 1.7 cm in diameter. Consequently, the ratio of depth to width of this distributor is larger than would be desired. As a result some caution must be taken when column efficiencies are evaluated due to the relatively high increase in extra pre-column volume. Consequently, the focus of this work is the process of sample transport across the column radially, more so than the axial dispersion.

The photographs in Fig. 2 compare the sample entry of the iodine through (a) the 10 μm frit (b) the 2 μm frit and (c) the distributor. Of these photographs, the sample entry through the 2 μm frit is clearly the most parabolic, while the sample entry through the distributor appears to be uniform and equally distributed across the entire column cross section. The best sample/flow distribution was undoubtedly obtained through the 10 μm frit. The photographs in Fig. 3 illustrate the sample entry though the distributor in more detail. Fig. 3a–c show the entry into the column and subsequent initial migration while Fig. 3d verifies the symmetry of the band from a photograph recorded at right angles to that in Fig. 3c. (Detailed photographs of the sample entry through the 2 μm frit and the 10 μm frit were presented previously and need not be shown here

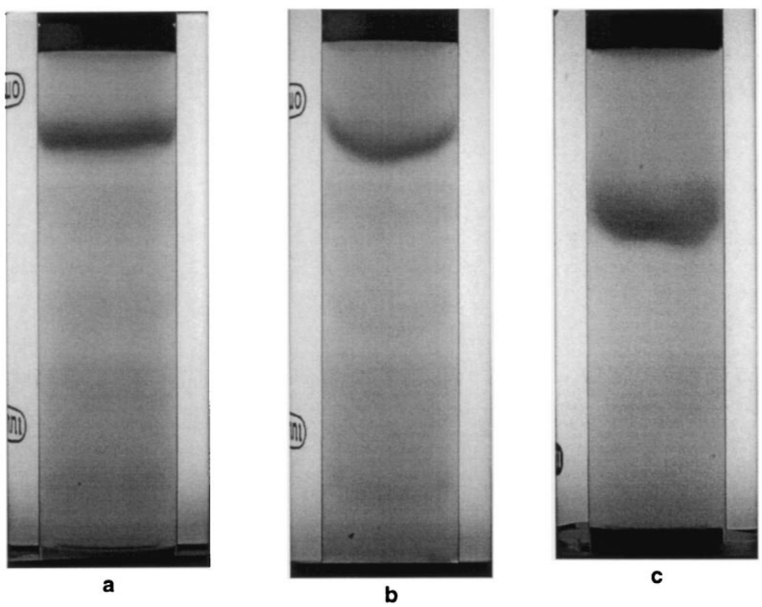

Fig. 2. Photograph of the sample band having cleared the inlet of the column which contained (a) a 10 μm frit, (b) a 2 μm frit and (c) the distributor. Flow rate=1.5 ml/min.

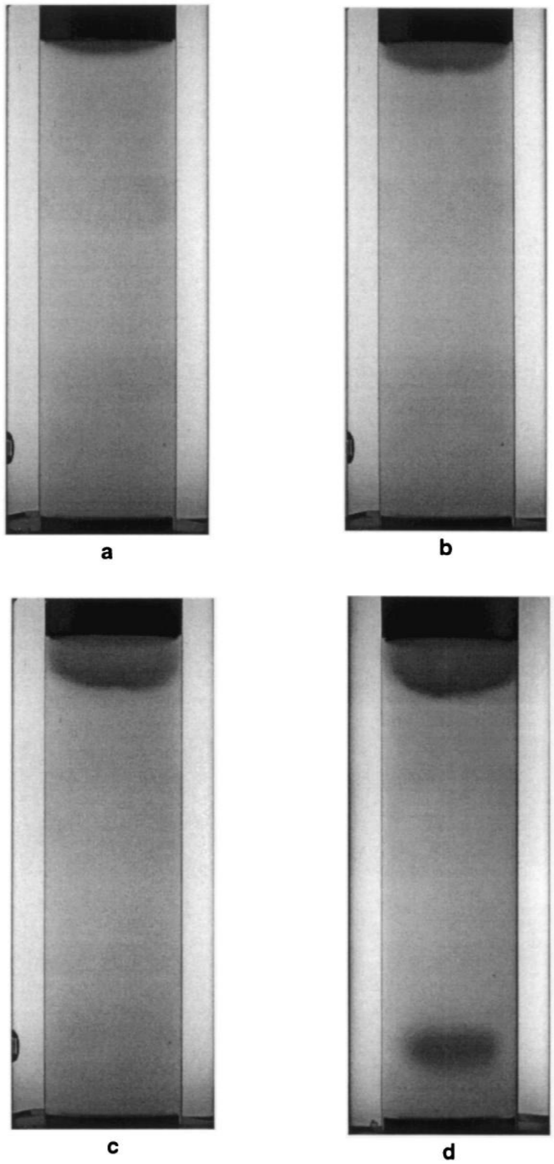

Fig. 3. Photographs detailing the sample entry through the distributor, (c) and (d) were taken at right angles.

[1]). Evaluation of the migration zones resulting from sample distribution through the distributor revealed that the central region of the band traveled faster than the wall region. This is shown by evaluating the on-column migration profiles obtained at various sections across the column [1–3]. Fig. 4a represents the left-hand side (LHS) of the column containing the distributor while Fig. 4b represents the right-hand side (RHS) of the same column. Clearly the central region of the band migrated at a faster rate. For ease of comparison we also present the LHS migration profiles for each of the columns containing the 10 μm frit and the 2 μm frit (Fig. 4c and d, respectively) The migration profiles of the 10 μm frit were coincident, while those of the 2 μm frit displayed slightly more variation than those of the distributor. However, an additional contribution to axial dispersion was caused by the higher extra-column void volume of the distributor, resulting in a wider sample band.

A more exact comparison of the migration zones may be made by evaluating the normalized migration distances. Fig. 5 illustrates these normalized migration distances where the rate of migration relative to the central band for each of the 10 μm frit (curve a), the 2 μm frit (curve b) and the distributor (curve c). Undoubtedly, the most uniform flow velocity was obtained through the 10 μm frit. The flow through the distributor was not entirely symmetrical. The sample on the RHS of the column migrated faster than the sample on the LHS, but even so the flow distribution was superior to that of the 2 μm frit with respect to the distribution along the column axis. An overall less parabolic migration zone was obtained using the distributor, in comparison to the 2 μm frit.

In addition to a header providing uniform flow distribution across the column, the main role of a distributor is in fact to provide a uniform sample distribution across the column radially. Fig. 6 illustrates the concentration distribution of sample for each of (a) the 10 μm frit, (b) the 2 μm frit and (c) the distributor. The normalized area represents the area under the concentration curves for each section of the column relative to the area of the central band. The results in Fig. 6 illustrate that the distributor performed poorly in respect to sample distribution. The LHS of the column contained a high concentration while the RHS contained a low concentration. In comparison, both the 2 μm frit and 10 μm frit distributed the sample in at least an almost symmetrical distribution, albeit not uniformly. This result is somewhat surprising and disturbing. A chromatographer, in good faith, employs the services of a distributor so that the sample is loaded uniformly onto the head of the column, but this appears to not be the case in this instance.

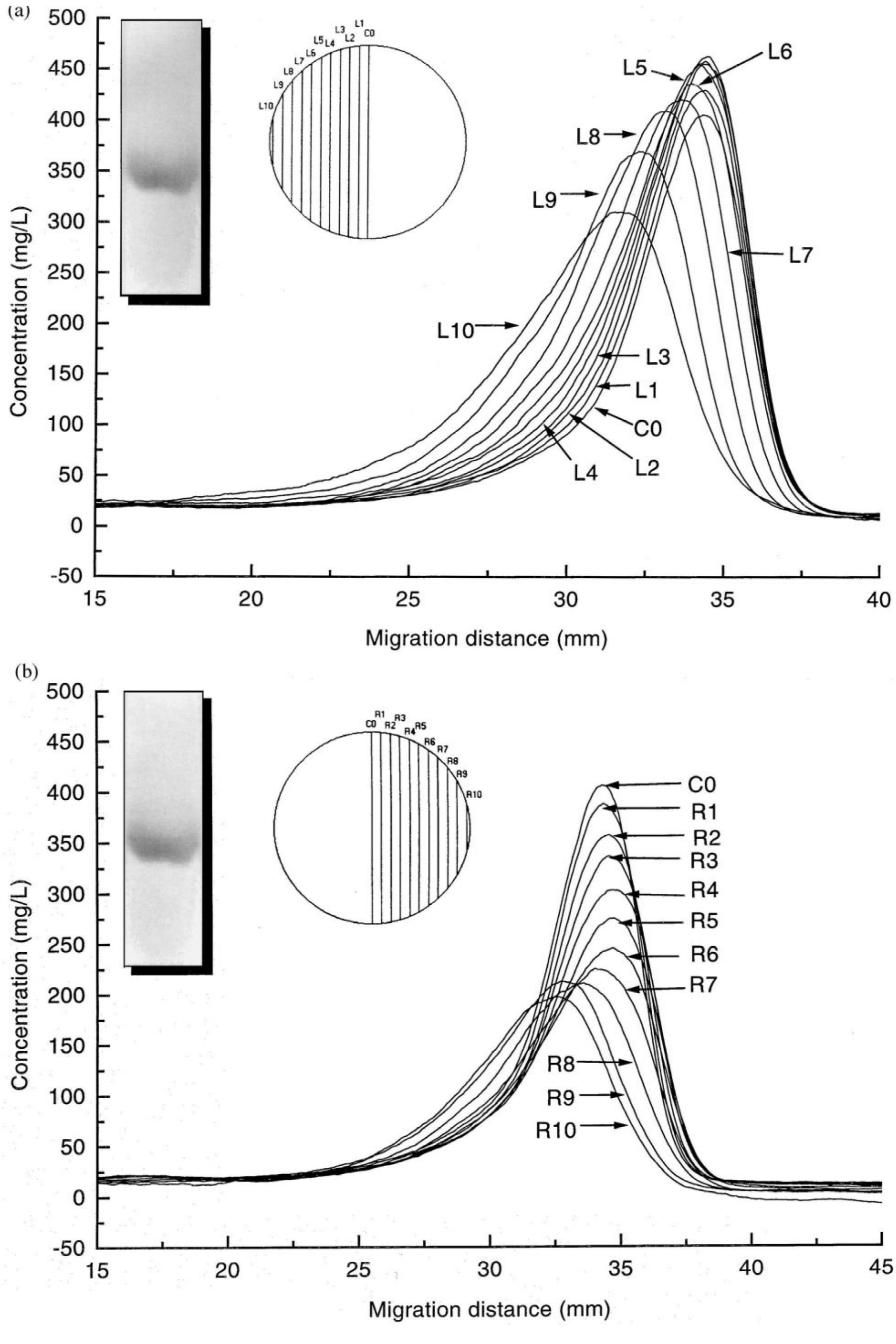

Fig. 4. Migration band profiles. (a) and (b) were obtained from the profiles measured over the sample band illustrated in Fig. 3. (a) Represents the left-hand side and (b) represents the right-hand side. (c) Represents the LHS migration profiles from the profiles measured over the sample from Fig. 2a, while (d) represents the LHS migration profiles from the histograms measured over the sample from Fig. 2b. Each band represents a surface of 0.32 mm (approximately 15 particle diameters) The image was divided into 21 or 19 sections radially across the column.

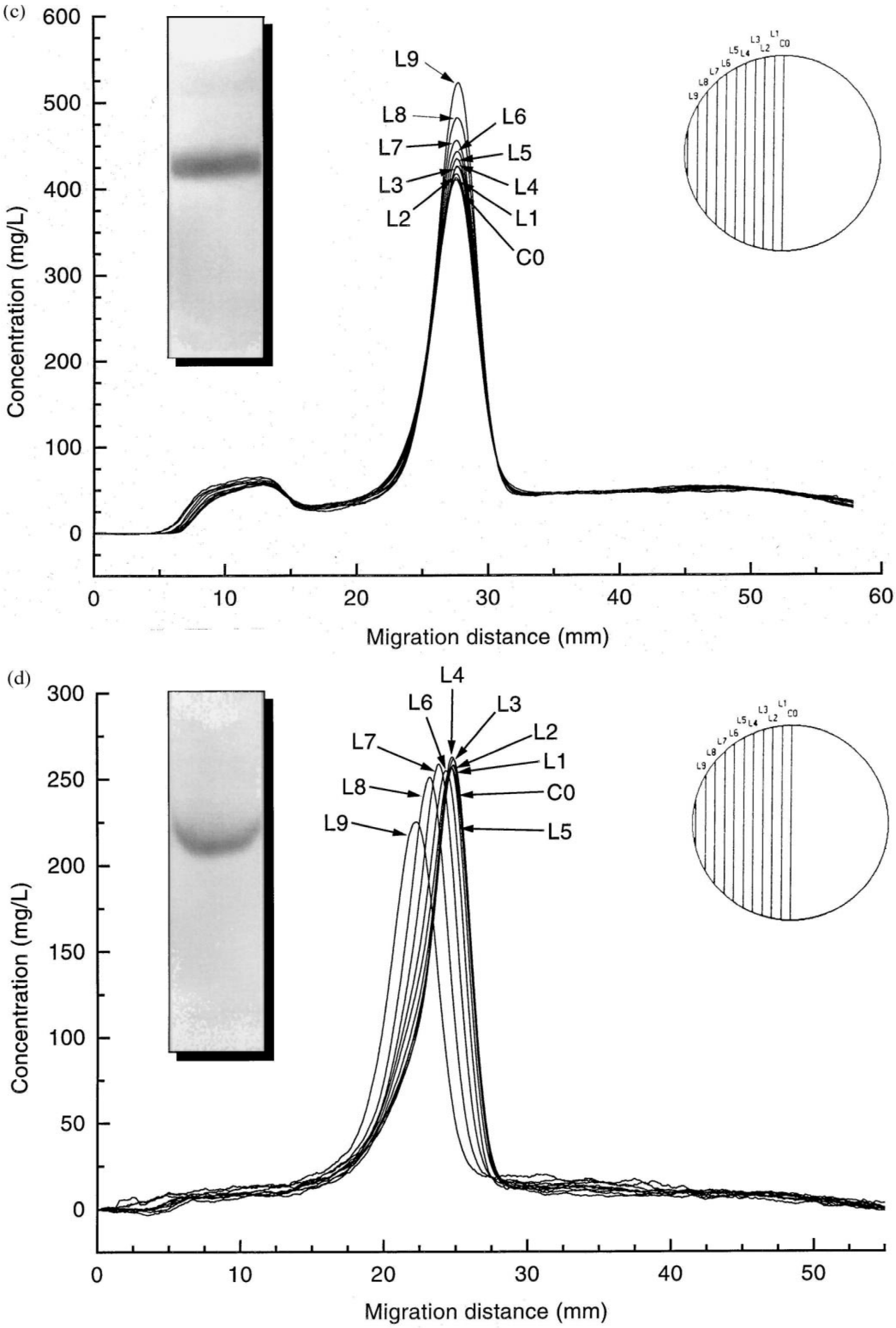

Fig. 4. (*continued*)

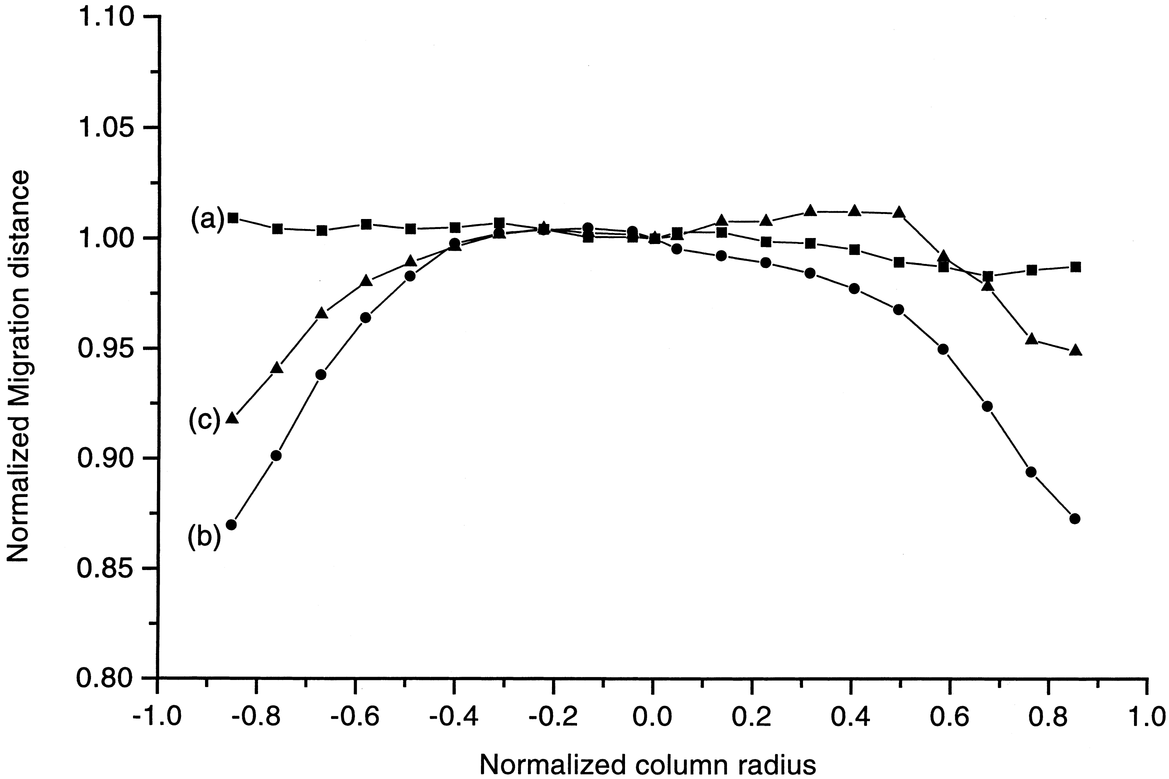

Fig. 5. Plots of normalized migration distances relative to the central band for each of (a) the 10 μm frit (b) the 2 μm frit and (c) the distributor.

Perhaps an explanation for this behavior can be found by evaluating the flow stream as it enters into an empty section of tubing. A stream of iodine was pumped into a column containing only carbon tetrachloride. The inlet fitting was a 2 μm frit. In this instance, an extremely narrow stream of iodine passed through the frit, apparently undisturbed (despite the fine pore size of the frit) and then proceeded to hit the frit at the column outlet, at which time a large amount of back flow was observed. Considerable dilution took place with turbulent mixing of the plug. This process is illustrated in the series of photographs in Fig. 7. In this instance the flow-rate was 1.0 ml/min (hence the Reynolds number for this flow if it were assumed to be a Poiseuille flow would be 2.0), but the same phenomena was observed even at flow-rates as low as 0.2 ml/min. A similar situation may be occurring within the distributor due to the coarse nature of the inlet side (hence a large void). Consequently, by the time the sample impinges into the finer region of the distributor, sample back mixing has already occurred and consequently the sample distribution is poor and axial dispersion is more significant. Close inspection of the sample injection also verifies that the sample enters the column through a series of pulsations rather than a continuous flow stream, further adding to the heterogeneity of the injection plug. Such pulsation only serves to make the distribution of the sample a more difficult problem. Although the photographs in Fig. 7 do not clearly illustrate this pulsation, the sample injection was captured on video, and at a flow-rate of 0.2 ml/min the pulsation is clearly visible [16].

The performance of the distributor improved when used in combination with a frit (both a 2 μm frit or a 10 μm frit). A slight improvement in the sample migration profile eluting through a 2 μm frit was also observed when the distributor was employed, although these improvements were marginal (Fig. 8).

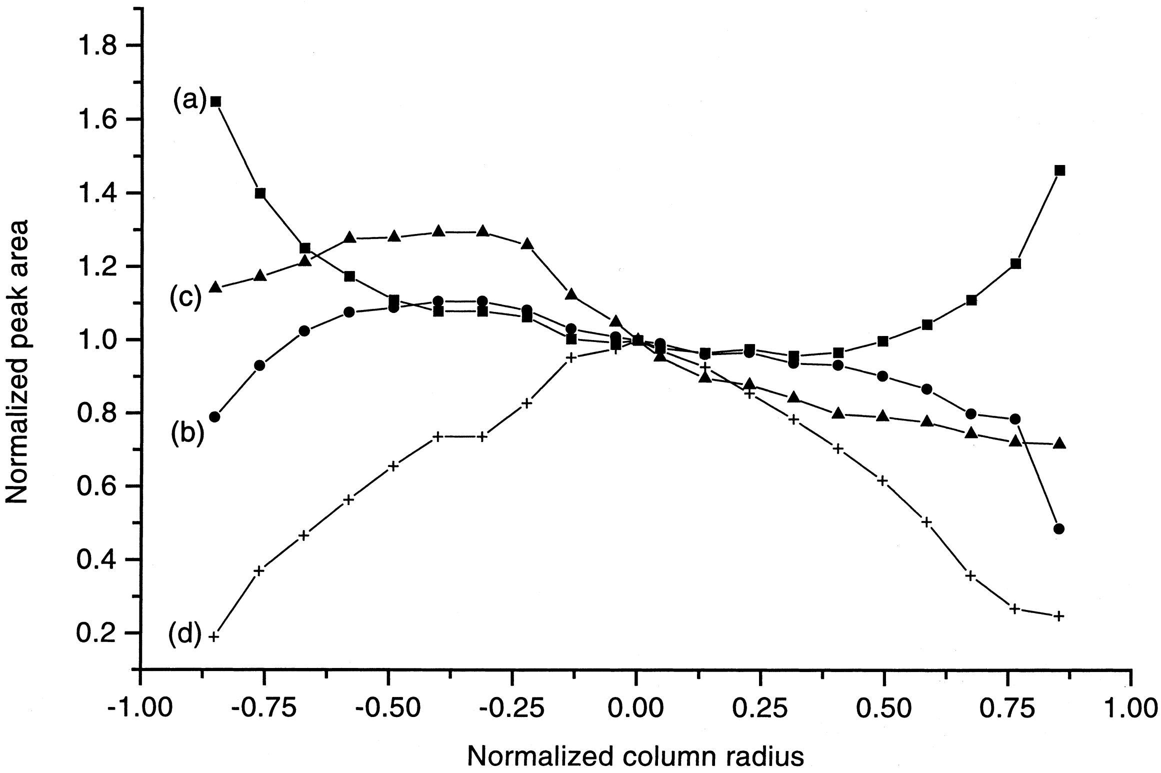

Fig. 6. Plot of the sample concentration distribution versus the radial location for each of the columns containing (a) the 10 μm frit, (b) the 2 μm frit (c) the distributor and (d) the distributor and the 2 μm frit.

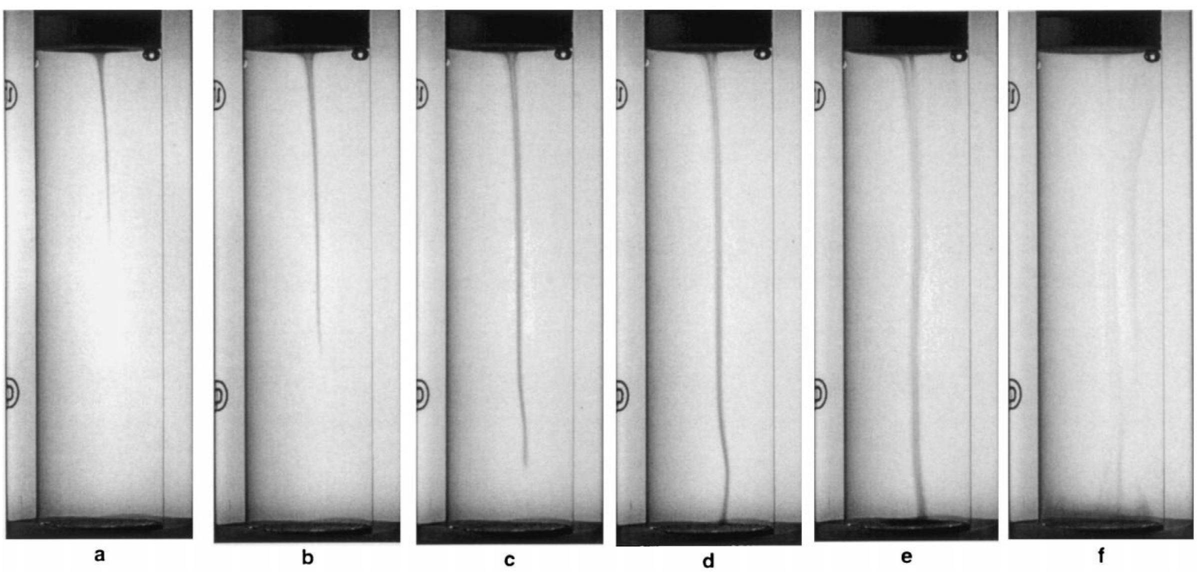

Fig. 7. A series of photographs depicting the sample entry into a column containing no stationary phase. The column contained only carbon tetrachloride. Total elapsed time <30 s.

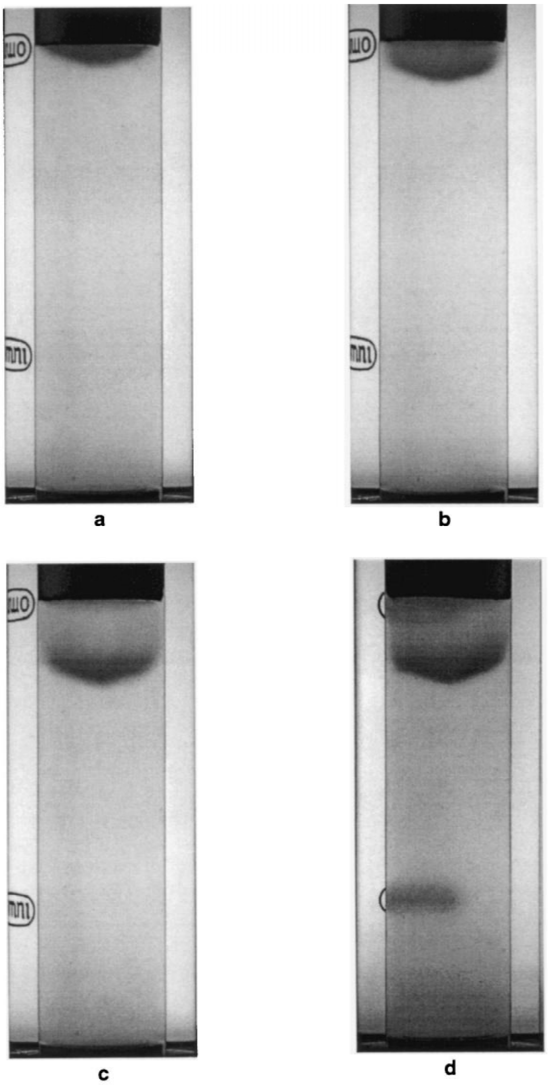

Fig. 8. (a–c) Photographs of the sample entry through the distributor and the 2 μm frit. (d) Photograph of the same sample at right angles to that of (c). Flow rate=1.5 ml/min.

The resulting migrating profiles extracted from these photographs are illustrated in Fig. 9. It is perhaps the fact that less sample was distributed to the wall that makes these migration profiles more pleasing to the eye. However, the fact that less sample was distributed to the wall when the distributor was used in conjunction with the 2 μm frit is somewhat disturbing, as shown in Fig. 6 (curve d). While the sample distribution through the distributor and 2 μm frit was more uniform than the distributor alone, it was far worse than for the 2 μm frit without the distributor. The sample entered the column in the central region of the bed and comparatively very little sample was distributed to the wall.

Fig. 10 illustrates a plot of local column efficiencies at various radial locations for each of (a) a column containing the 10 μm frit, (b) the 2 μm frit, (c) the distributor, (d) the distributor and the 10 μm frit and (e) the distributor and the 2 μm frit. The reduced plate height was measured using the half height method. The efficiency measurements for both columns containing the 10 μm frit and the 2 μm frit showed that both had very efficient and uniform beds, while the column containing the distributor was less efficient with greater variation across the column. For this column, the central region was the most efficient and the region nearest the wall was the least efficient. The bed was, however, symmetrically uniform. While we do not wish to place too much emphasis on the column efficiencies measured from each of the different head fittings, it is interesting to note that the performance of the distributor improved when used in conjunction with a frit, either the 2 μm frit or the 10 μm frit, with the improvement being greatest for the finer pore size. Even so, the efficiency measurements for the region near the wall (extending out from approximately 0.75 r) showed that the column behaved poorly in this region. However, evaluation of the results in this region of the column must be viewed with caution, as only a very low quantity of sample was distributed to the wall when the distributor was employed. Consequently, the validity of an accurate measurement of band width is less reliable.

In concluding we must comment on the experimental procedure. Because of the nature of the glass column and the design of the head fitting a new column was necessary for each experiment. This meant that the distributor could not be used on the exact same column as was used for either of the frits etc. Consequently, column to column variation could be high, negating to some degree the conclusions. However, we must remember that we are testing the role of a distributor. The primary function of which is to provide a uniform sample distribution across the column. This to the most part does not depend on the column, but rather the inlet. In this respect, the

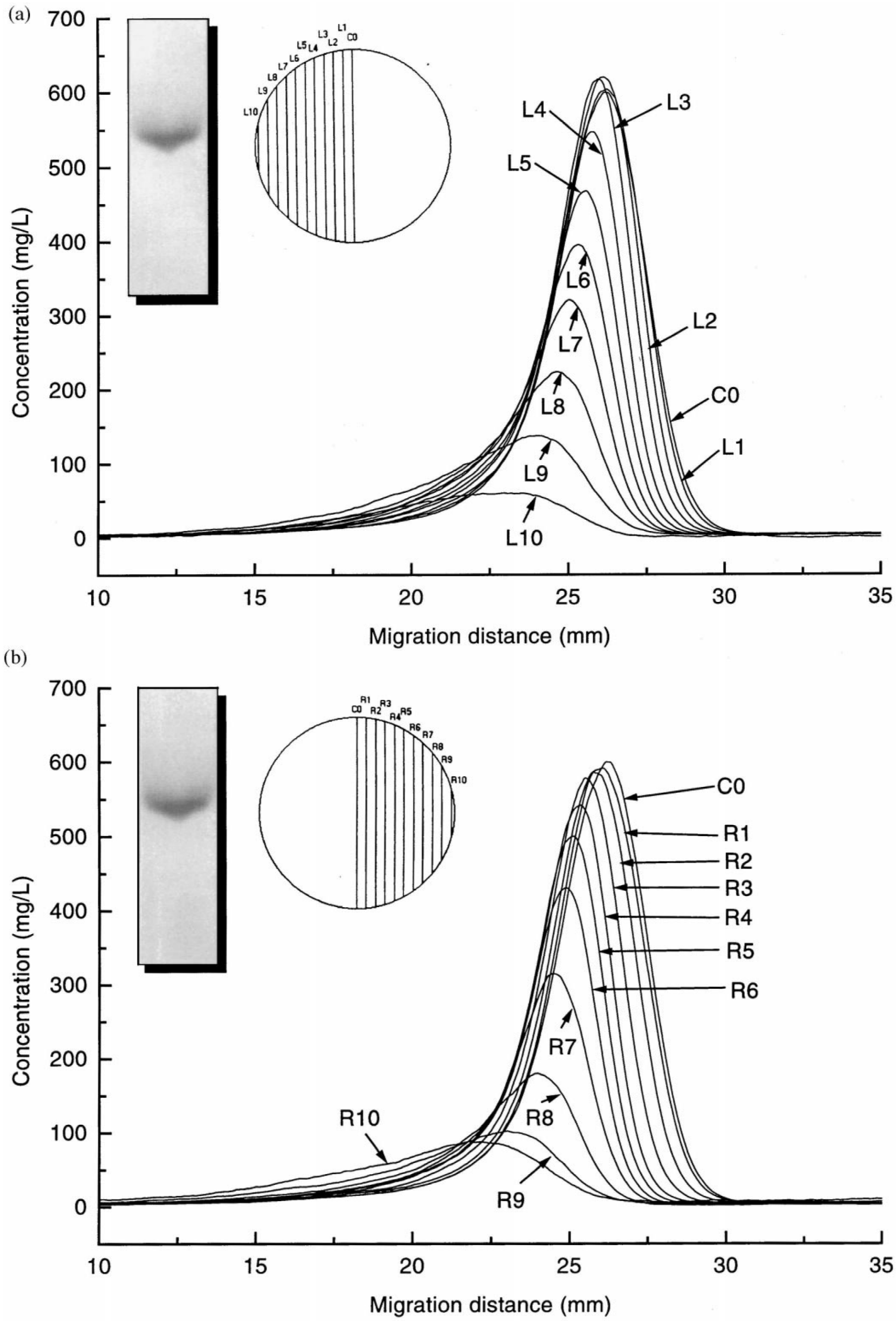

Fig. 9. Migration band profile of the sample illustrated in Fig. 8. (a) Represents the left hand side and (b) represents the right hand side.

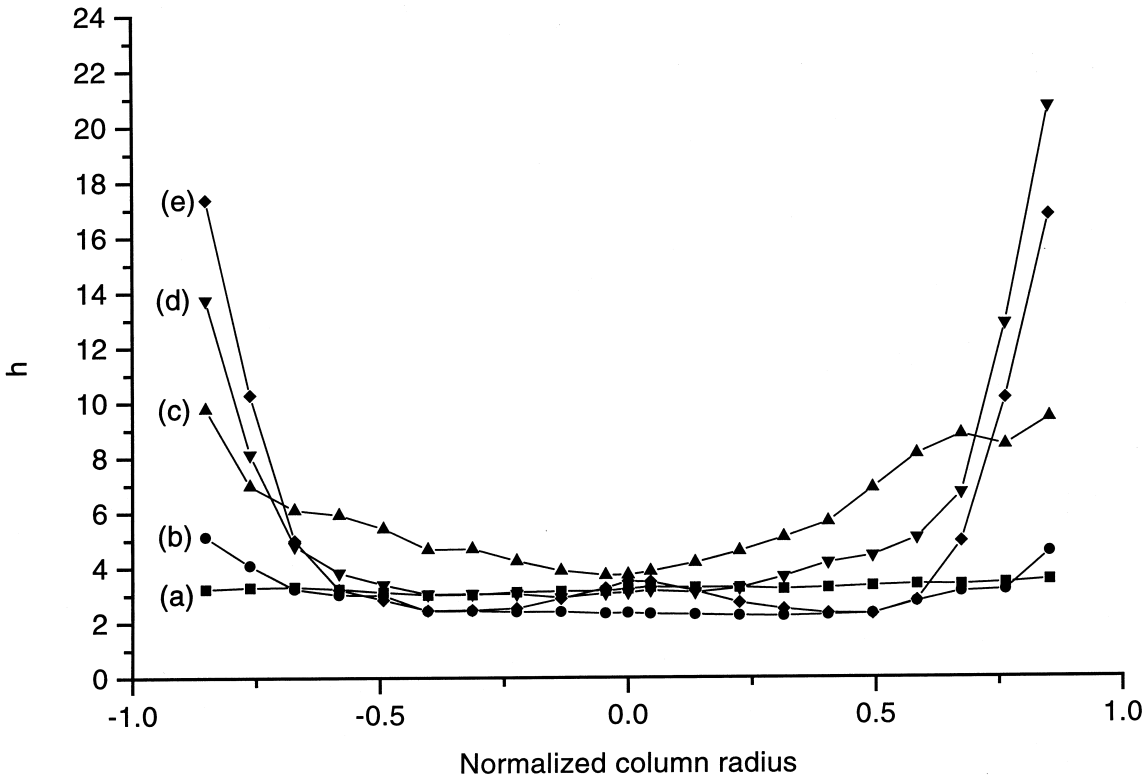

Fig. 10. Plot of local column efficiencies at various radial locations for columns containing (a) the 10 μm frit, (b) the 2 μm frit, (c) the distributor, (d) the distributor and the 10 μm frit and (e) the distributor and the 2 μm frit. The reduced plate height was measured using the half height method.

distributor behaved poorly. The sample was concentrated mainly in the central region of the column, and also the distribution was not symmetrical about the column axis. In comparison, the results for each of the frits showed that the sample distribution was at least almost symmetrical, if not uniform.

The column efficiency gave us a measure on the bed performance for each column. All the columns prepared had similar efficiencies in the central region. The columns containing the 10 μm frit and the 2 μm frit were homogeneously uniform with respect to efficiency. Likewise, when the distributor was used in conjunction with a frit, the homogeneity of the bed improved in comparison to the heterogeneity observed for the distributor alone. We believe that the fall in efficiency near the wall region observed whenever a distributor was employed is as much to do with the poor sample distribution to the wall region as it is to do with the variation in flow distributions that were observed. This was certainly not the purpose of adding a distributor at the column inlet. Clearly, the inlet geometry and the flow pattern resulting from this geometry plays an important role in establishing the apparent column homogeneity.

4. Conclusion

The first conclusion of this study is that one should always be conscious that the addition of a distributor of this type ahead of the column may have some detrimental effects on the column performance. It should be decided carefully. It was nefarious in connection with the 10 μm frit but provided a more uniformed flow distribution when used with the 2 μm frit, albeit, the sample distribution was poorer. Consequently, the migration profile was tailing less than with the 2 μm frit alone. This improvement was not reflected in the column efficiency because the overall band width increased – a probable consequence of the rather large volume of the distributor. Admittedly, the variance contribution of the distributor should decrease when the effective column length increases. It is stronger on the profiles recorded along the column after a short time, as is the case in the present study, than in elution profiles at the end of a longer column.

Accordingly, it seems better to optimize the frit porosity to the packing material used and, for analytical columns at least, dispense with the use of a distributor. For columns that require the services of a small pore-size frit, or for larger diameter columns, addition of a distributor could improve the shape of the sample band entering the column, but at the expense of an additional contribution to axial dispersion. If the column volume was large in comparison to the distributor volume, this increased band spreading could be tolerated. A disturbing aspect of the distributor effect, however, was the variation that it seemed to cause in the radial column efficiency. We do not know enough yet regarding the behavior of distributors nor the radial distribution of their own permeability which is probably largely influenced by their design and manufacturing process. Further investigations of this problem are needed.

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